Serum-free generation of multipotent mesoderm (kdr(+)) progenitor cells in mouse embryonic stem cells for functional genomics screening.

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Public Summary:
A major promise of stem cells is their use in high throughput screening for the discovery of potential drug targets and drugs themselves. Here we present a protocol for the functional screening of microRNA and siRNA libraries to uncover key proteins that control differentiation of stem/progenitor cells into cardiomyocytes.

Scientific Abstract:
This unit describes a robust protocol for producing multipotent Kdr-expressing mesoderm progenitor cells in serum-free conditions, and for functional genomics screening using these cells. Kdr-positive cells are able to differentiate into a wide array of mesodermal derivatives, including vascular endothelial cells, cardiomyocytes, hematopoietic progenitors, and smooth muscle cells. The efficient generation of such progenitor cells is of particular interest because it permits subsequent steps in cardiovascular development to be analyzed in detail, including deciphering the mechanisms that direct differentiation. In addition, the oligonucleotide transfection protocol used to functionally screen siRNA and miRNA libraries is a powerful tool to reveal networks of genes, signaling proteins, and miRNAs that control the diversification of cardiovascular lineages from multipotent progenitors. Technical limitations, troubleshooting, and potential applications of these methods are discussed. Curr. Protoc. Stem Cell Biol. 23:1F.13.1-1F.13.13. (c) 2012 by John Wiley & Sons, Inc.